NOA81 was pre-cured with well-defined spacers (~360 μm) placed between a glass slide (plasma treated) and PDMS working stage under collimated UV light source (365 nm, ~8.3 mW/cm^2) through a photomask for 5 seconds. After limited exposure, the glass slide was slowly removed from the PDMS slab and a syringe is used to wash out the unexposed adhesive regions by injecting with ethanol, ethanol/acetone mixture (1:1) and ethanol again. The air-dried glass slide was post-cured by flood exposure under UV light for 345 sec to increase the adhesion to glass, followed by a 12 h thermal cure at 50° C. to extend the structure's lifetime. Thereafter, the Master-1# was treated by vapor deposition (dimethyl dichlorosilane) and utilized to mold PDMS (Sylgard 184, Dow Corning, with a 10:1 mass ratio of base to curing agent). The PDMS substrate was cured at 75° C. for 4 hours and peeled off; 3-mm holes were punched with a 9 mm pitch between centers to form Master-2# (FIG. **1**C-**2**). After attaching PDMS Master-2# on a plasma-treated glass slide, NOA81 was injected into the channels and wells without bubble trapping, cured under adequate UV exposure dose (1300 sec) and thermally aged at 50° C. for 12 hours. By carefully removing Master-2#, the final Master-3#, including both lower channel features (~360 μm) and higher pillar structures (~4.6 mm), was accomplished (FIG. **1**C-**3**) and can then be used for replication of PDMS substrate with pre-formed holes (FIG. **1**C-**4**).

[0060] Magnetic droplet manipulation. A syringe pump (Model: NE-300, New Era Pump Systems Inc.) based handling stage was used to perform M2 chip-based automatic manipulation of multiple magnetic droplets (FIG. **2**A). Prior to use, the M2 chip was treated with silanizing agent and then loaded with M5904 mineral oil (Sigma) and buffers/reagents at designated wells with multichannel pipette. A permanent neodymium magnet (1" cube) was fixed between two guide rods of the syringe pump. The primed M2 chip was put on top of the magnet, attached on the pusher block of the syringe pump by using adhesive tape and held up on the right side by a home-made plastic support, which was assembled on the guide rods. With the pusher block moving from left to right at a velocity of ~1 mm/sec, the M2 chip moves as well, but the SMPs (1 μm diameter) in microwells (submerged in mineral oil) are retained and collected at the edge area of the magnet because of the large gradient of the magnetic field there, causing relative movement of SMPs to M2 chip from right to left. FIG. **2**B shows the process of M2 chip-based SMPs manipulation. Pictures 11~14) show the process of separating and retaining SMPs from aqueous droplets. Four small droplets containing SMP (SMP-1) were split from parent droplets (with red dye), merged with each other and finally trapped in the collection well. Pictures 21~24) represent the process of SMP-2 passing through a tapered channel (video-2 in ESI†), moved from wash well (water droplet) to elution well (droplet with blue dye).

[0061] MicroRNA purification. MicroRNA purification from biological samples is based on the fact that small RNA molecules (including miRNAs) will selectively bind to silica surfaces in the presence of 70% ethanol and a chaotropic salt (User manual for PureLink miRNA Isolation Kit, Invitrogen). Lysis/binding buffer (L3) and washing buffer (W5) was used from a commercial kit (PureLink miRNA Isolation Kit, Invitrogen) to obtain cell lysis and miRNA purification. Cultured cell pellets were resuspended in L3 buffer and mixed well by vortexing. The cell lysate or miRNA sample was diluted using L3 buffer to a proper concentration as a load sample. Prior to injection into the M2 chip, the SMPs were washed twice with nuclease-free water from Sigma-Aldrich (St. Louis, Mo.) and then resuspended in ethanol (>95%) to a specific concentration according to the application.

[0062] For the miRNA extraction, a suspension (1.35 μL) of SMPs (SMP-1, 100-140 ug) in ethanol was added into a sample droplet (2.5 μL) in the binding well to obtain a solution of 35% ethanol. This solution was further mixed by pipetting up and down 10 times and was then incubated for 4 min. Large nucleic acid molecules adsorbed to the SMP-1 surface while small RNA molecules remained in the solution. Thereafter, a small droplet containing the SMP-1 with the adsorbed large DNA/RNA molecules was split from the sample droplet and finally collected and trapped in the collection well (FIG. **3**A). To facilitate the selective binding of small RNA molecules to SMPs, another suspension (4.5 μL) of SMP-2 (60-80 μg) in ethanol was added into the sample droplet to give a solution of 70% ethanol (FIG. **3**B). Small RNA molecules bound to the SMP-2 and impurities were diluted and washed away by passing the splitted SMP-2 droplet through three W5 buffer droplets (10 μL, 30 sec each) successively. Finally, the adsorbed small RNA molecules were eluted to a Sigma water droplet (2.5 μL for in-tube RT-qPCR or 0.915 μL for on-chip RT reaction) in elution well. The small RNA-desorbed SMP-2 droplet was removed from the elution well and moved to the collection well (FIG. **3**C) in the end. The total miRNA purification process, from the sample injection to elution, takes less than 15 min.

[0063] As a comparison, a silica-membrane spin column-based miRNA isolation kit (PureLink, Invitrogen) was run in parallel with the M2 chip for miRNA purification from cell lysate. 100 μL of cell lysate sample diluted in L3 buffer was loaded into the spin column and the purified small RNAs was eluted to 100 μL of Sigma water.

[0064] MicroRNA RT-qPCR detection. TaqMan microRNA RT kit (Cat. No. 4366596) and primers (Cat. No. 4427975) were purchased for hsa-mir-191 from Ambion (Austin, Tex.). Taq 5×Master Mix for PCR was from New England BioLabs (Cat. No. M0285S). For off-chip real time RT-PCR, 7.5 μL of RT reaction mixture contained 2.5 μL of purified miRNA and 5 μL of RT pre-mix including 1×RT Buffer, 1 mM of dNTP mixture, 25 U of Multiscribe RT enzyme, 2 U of RNase Inhibitor and 1.5 μL of 5×RT stem-loop primers. RT reaction was performed at 16° C. for 30 min, 42° C. for 30 min and 85° C. for 5 min. Thereafter, 0.67 μL of RT product was added into 9.33 μL of PCR pre-mix, containing 2 μL of Taq 5×Master Mix, 0.5 μL of 20×TaqMan probe and primers. The reaction was run on the BioRad CFX96 real-time PCR detection system at 95° C. for 3 min, followed by 45 cycles (95° C. for 15 s and 60° C. for 1 min).

[0065] As for on-chip reactions, 0.585 μL of RT pre-mix was added to the elution buffer droplet (0.915 μL) after elution process. Following on-chip RT reactions, the RT product was mixed with 6 μL of PCR pre-mix (including 0.1% bovine serum albumin, BSA to prevent possible enzyme adsorption and improve PCR efficiency) off-chip or on-chip, to perform either real-time PCR for cell lysate analysis or chip-PCR and end-point detection to test the potential of on-chip integration of SPE and RT-PCR. Temperature control was realized by using a regular PCR machine with a 0.4 mm-thick brass plate placed under the M2 chip to enhance heat transfer and temperature uniformity. Mineral oil and small pieces of PDMS spacer was applied to ensure fully thermal contact of the M2 chip to the heating plate. To adjust the set temperatures and